

# INTERNATIONAL COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents  
United States Patent and Trademark  
Office  
Box PCT  
Washington, D.C. 20231  
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year)

03 February 2000 (03.02.00)

International application No.

PCT/JP99/02683

Applicant's or agent's file reference

99-F-032PCT

International filing date (day/month/year)

21 May 1999 (21.05.99)

Priority date (day/month/year)

22 May 1998 (22.05.98)

Applicant

LUKACSOVICH, Tamas et al

1. The designated Office is hereby notified of its election made:



in the demand filed with the International Preliminary Examining Authority on:

17 December 1999 (17.12.99)



in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was



was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Maria Kirchner

Telephone No.: (41-22) 338.83.38





REC'D 18 AUG 2000

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## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 99-F-032PCT	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/JP99/02683	International filing date (day/month/year) 21/05/1999	Priority date (day/month/year) 22/05/1998
International Patent Classification (IPC) or national classification and IPC C12N15/10		
Applicant JAPAN SCIENCE AND TECHNOLOGY CORPORATION et al.		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 5 sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"><li>I <input checked="" type="checkbox"/> Basis of the report</li><li>II <input type="checkbox"/> Priority</li><li>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</li><li>IV <input type="checkbox"/> Lack of unity of invention</li><li>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</li><li>VI <input type="checkbox"/> Certain documents cited</li><li>VII <input type="checkbox"/> Certain defects in the international application</li><li>VIII <input type="checkbox"/> Certain observations on the international application</li></ul>		
Date of submission of the demand  17/12/1999	Date of completion of this report  14.08.2000	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer  Marinoni, J-C  Telephone No. +49 89 2399 8563 	



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/JP99/02683

**I. Basis of the report**

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

**Description, pages:**

1-21 as originally filed

**Claims, No.:**

1-19 as originally filed

**Drawings, sheets:**

1/7-7/7 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☐ the claims, Nos.:  
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/JP99/02683

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**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes:	Claims	1-19
	No:	Claims	NONE
Inventive step (IS)	Yes:	Claims	NONE
	No:	Claims	1-19
Industrial applicability (IA)	Yes:	Claims	1-19
	No:	Claims	NONE

**2. Citations and explanations**

**see separate sheet**





**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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International application No. PCT/JP99/02683

Reference is made to the following document:

- D1:** BIOTECHNOLOGY, VECTORS A SURVEY OF MOLECULAR CLONING VECTORS AND THEIR USES; R. L. RODRIGUEZ AND D.T. DENHARDT, Vol. 1, 1988, pages 437-456, Butterworths, Boston, Pirotta 'Vectors for P-mediated transformation in *Drosophila*'
- D2:** DEVELOPMENT, Vol. 118, pages 401-415, 1993, Brand & Perrimon 'Targeted gene expression as a means of altering cell fates and generating dominant phenotypes'

The following document was not cited in the search report. A copy is appended herewith:

- D3:** NATURE, Vol. 392, 9 April 1998, pages 608-611, Zambrowicz et al. 'Disruption and sequence identification of 2,000 genes in mouse embryonic stem cells'

**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. The general strategy called enhancer trapping and vectors useful in its application are well-known in mouse cells and drosophila (for drosophila, see **D1** for example). The vectors therein disclosed allow, after they are incorporated into the drosophila genome, to characterize sequences surrounding the insertion site. Recent advances and improvement of the technique in mouse have led to gene trapping vectors (see **D3**, especially figure 1).  
**D3** discloses a vector for gene trap that contains in this order, a splice acceptor sequence, a  $\beta$ gal/neomycine phosphotransferase fusion gene (*i.e.* a reporter/drug resistance gene), a puromycin N-acetyl transferase gene (*i.e.* a gene responsible for a detectable phenotype) and a splice donor sequence. This vector is useful for gene trap in mouse for the following reasons:
  - (i) not only does the vector get inserted within an intron and allow the production of a fusion protein encoded by the DNA sequences upstream the insertion site and the reporter gene (like other well known vectors previously used for gene trap in mouse cells)
  - (ii) but also the vector allows the production of another fusion protein encoded by a second reporter gene and the DNA sequences downstream the insertion



site thanks to a second promoter and a splice donor site.

Therefore, the two corresponding cDNAs can be sequenced resulting in the rapid identification of the full length sequence of the gene in which the transposable element is inserted.

The vector as presently claimed merely consists in the application of the features of the vector of **D3** to a vector useful for gene trap in drosophila, *i.e.* a vector modified to include markers and characteristics allowing the method to work in drosophila. All these characteristics (GAL4 as reporter gene, mini-white as detectable phenotype, use of heatshock promoters, etc...) are commonly used in gene trapping in drosophila (see **D1** and **D2** for example).

The skilled person would then successfully adapt the vector of **D3** to the specificity of drosophila by replacing the selection markers and reporter genes with well-known markers and reporter genes used in gene-trap methods in drosophila.

Therefore, **claim 1** but also **claims 9, 10 and 16** do not meet the requirements of Article 33(3) PCT concerning inventive step.

2. None of the dependent claims contains any features which, in combination with the features of any of the claims they refer, meet the requirements of the PCT in respect of inventive step.



## PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>99-F-032PCT</b>	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/JP 99/ 02683</b>	International filing date (day/month/year) <b>21/05/1999</b>	(Earliest) Priority Date (day/month/year) <b>22/05/1998</b>
Applicant  <b>JAPAN SCIENCE AND TECHNOLOGY CORPORATION et al.</b>		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 5

☐ as suggested by the applicant. ☐ None of the figures.

☒ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/JP 99/02683

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/10 C12N15/62 C12N15/85 C12N15/90 C12Q1/68  
C07K14/435

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 20031 A (JARVIK JONATHAN W) 14 May 1998 (1998-05-14) the whole document ---	1-19
A	A.H. BRAND AND N. PERRIMON: "Targeted gene expression as a means of altering cell fates and generating dominant phenotypes" DEVELOPMENT, vol. 118, 1993, pages 401-415, XP000857179 THE COMPANY OF BIOLOGISTS, LIMITED, CAMBRIDGE, GREAT BRITAIN cited in the application the whole document --- -/--	1-19

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### ° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

3 December 1999

Date of mailing of the international search report

22/12/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Hornig, H





## INTERNATIONAL SEARCH REPORT

International Application No

PCT/JP 99/02683

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	W. WURST ET AL.: "A large-scale gene trap screen for insertional mutations in developmental mutations in developmentally regulated genes in mice" GENETICS, vol. 139, no. 2, February 1995 (1995-02), pages 889-899, XP000857167 GENETIC SOCIETY OF AMERICA, BALTIMORE, MD, US the whole document ---	1-19
A	C. WILSON ET AL.: "P-element-mediated enhancer detection: an efficient method for isolating and characterizing developmentally regulated genes in Drosophila" GENES & DEVELOPMENT, vol. 3, no. 9, September 1989 (1989-09), pages 1301-1313, XP000857178 CSH LABORATORY PRESS, NEW YORK, US cited in the application the whole document ---	1-19
A	P. BARTHMAIER AND E. FYRBERG: "Monitoring development and pathology of Drosophila indirect flight muscles using green fluorescent protein" DEVELOPMENTAL BIOLOGY, vol. 169, no. 2, June 1995 (1995-06), pages 770-774, XP002124662 ACADEMIC PRESS, INC., US the whole document ---	1-19
A	C.S. THUMMEL ET AL.: "Vectors for Drosophila P-element-mediated transformation and tissue culture transfection" GENE, vol. 74, 1988, pages 445-456, XP002124663 ELSEVIER SCIENCE PUBLISHERS, B.V., AMSTERDAM, NL; the whole document ---	
A	V. PIRROTTA: "Vectors for P-mediated transformation in Drosophila" BIOTECHNOLOGY, VECTORS A SURVEY OF MOLECULAR CLONING VECTORS AND THEIR USES; R. L. RODRIGUEZ AND D.T. DENHARDT, vol. 1, 1988, pages 437-456, XP000857168 Butterworths, Boston, US cited in the application the whole document ---	
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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/JP 99/02683

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>C.S. THUMMEL AND V. PIRROTTA: "New pCasPeR P element vectors"  EMBL SEQUENCE DATABASE,  23 July 1996 (1996-07-23), XP002124664  Cambridge, UK  Accession no. EMSYN.PEU59055; U59055;  &amp; DROS. INFO. SERVICE,  vol. 71, 1992, page 150  -----</p>	
A	<p>A. GOSSLER ET AL.: "Mouse embryonic stem cells and reporter constructs to detect developmentally regulated genes"  SCIENCE,  vol. 244, 1989, pages 463-465, XP002124665  AAAS, WASHINGTON, DC, US  cited in the application  the whole document  -----</p>	



INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/JP 99/02683

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9820031 A	14-05-1998	AU 5168598 A	29-05-1998





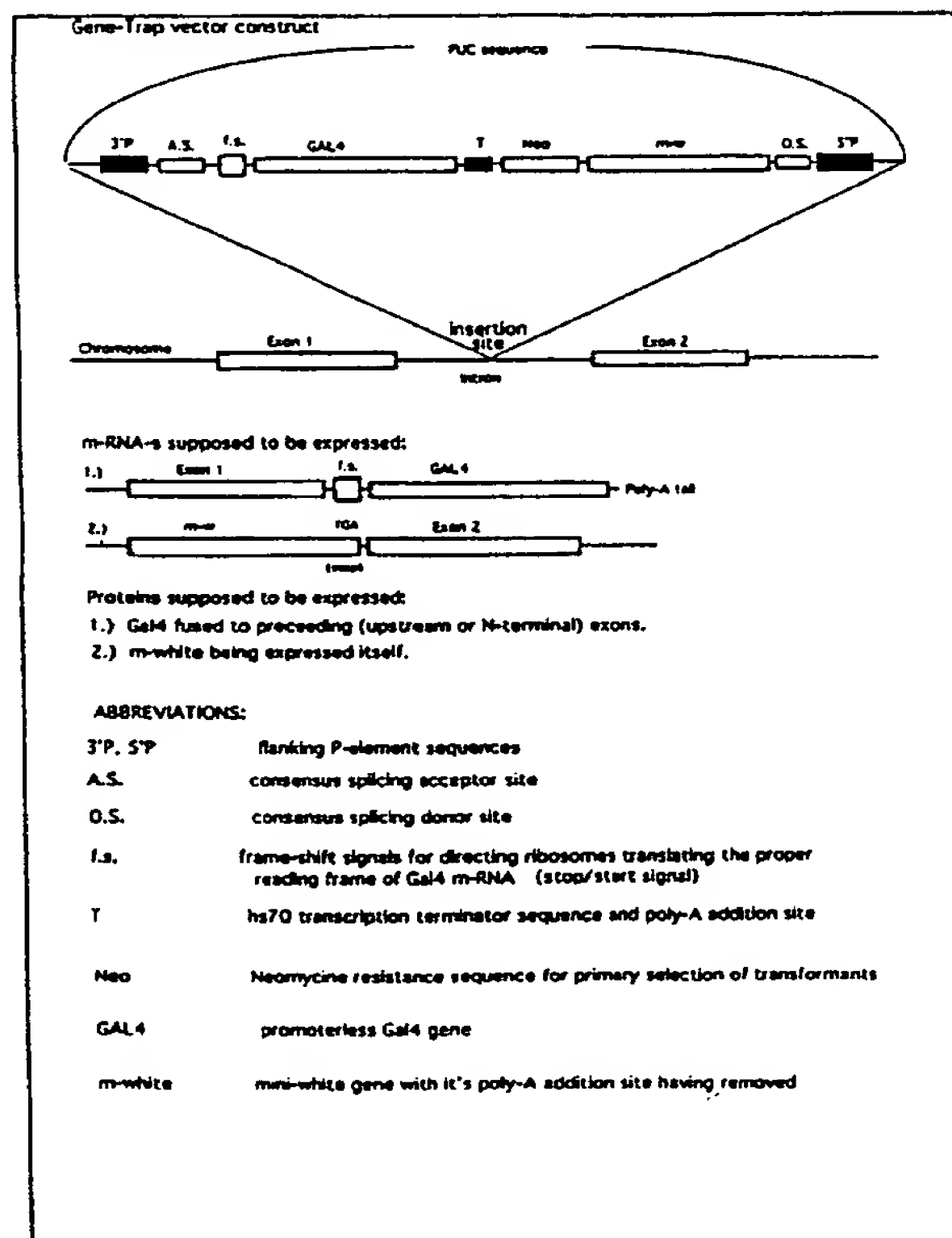
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C12N 15/10, 15/62, 15/90, C12Q 1/68, C07K 14/435</b>		A2	(11) International Publication Number: <b>WO 99/61604</b>
			(43) International Publication Date: 2 December 1999 (02.12.99)
(21) International Application Number: <b>PCT/JP99/02683</b>		(81) Designated States: CA, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(22) International Filing Date: 21 May 1999 (21.05.99)			
(30) Priority Data: 10/141952 22 May 1998 (22.05.98) JP		Published <i>Without international search report and to be republished upon receipt of that report.</i>	
(71) Applicant (for all designated States except US): JAPAN SCIENCE AND TECHNOLOGY CORPORATION [JP/JP]; 1-8, Hon-cho 4-chome, Kawaguchi-shi, Saitama 332-0012 (JP).			
(72) Inventors; and (75) Inventors/Applicants (for US only): LUKACSOVICH, Tamas [HU/JP]; 2-30-13, Narusedai, Machida-shi, Tokyo 194-0043 (JP). ASZTALOS, Zoltan [HU/JP]; 3-16-21, Narusedai, Machida-shi, Tokyo 194-0043 (JP). YAMAMOTO, Daisuke [JP/JP]; 4-18-8, Narusedai, Machida-shi, Tokyo 194-0043 (JP). AWANO, Wakae [JP/JP]; Famiyu-102, 3-10-12, Minamidai, Sagami-hara-shi, Kanagawa 228-0814 (JP).			
(74) Agent: NISHIZAWA, Toshio; 6F, Mani-Building, 37-10, Udagawa-cho, Shibuya-ku, Tokyo 150-0042 (JP).			

(54) Title: A VECTOR FOR GENE TRAP, AND A METHOD FOR GENE TRAPPING BY USING THE VECTOR

## (57) Abstract

The present application provides a vector for trapping an unknown gene of *Drosophila melanogaster*, which is a recombinant plasmid comprising the following nucleotide sequences in this order: an artificial consensus splicing acceptor site; a synthetic "stop/start" sequence; a reporter gene; a drug resistance gene; a gene responsible for a detectable phenotype of the *Drosophila melanogaster*; and a synthetic splicing donor site. The present application also provides a method for trapping an unknown gene of *Drosophila melanogaster* by using the vector.





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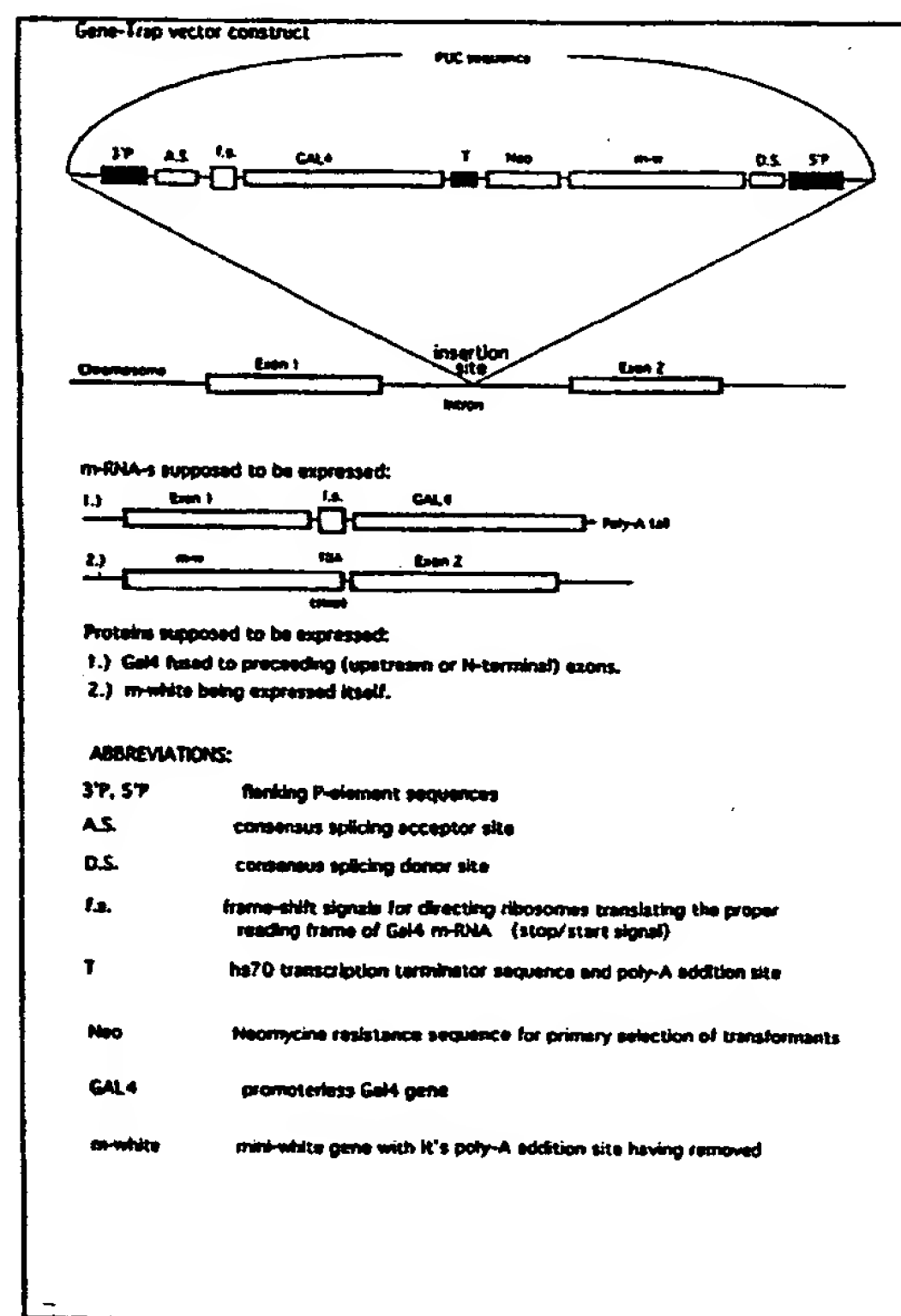
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C12N 15/10, 15/62, 15/85, 15/90, C12Q 1/68, C07K 14/435</b>		<b>A3</b>	(11) International Publication Number: <b>WO 99/61604</b>
			(43) International Publication Date: 2 December 1999 (02.12.99)
(21) International Application Number: PCT/JP99/02683		(81) Designated States: CA, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(22) International Filing Date: 21 May 1999 (21.05.99)			
(30) Priority Data: 10/141952 22 May 1998 (22.05.98) JP		Published With international search report.	
(71) Applicant (for all designated States except US): JAPAN SCIENCE AND TECHNOLOGY CORPORATION [JP/JP]; 1-8, Hon-cho 4-chome, Kawaguchi-shi, Saitama 332-0012 (JP).		(88) Date of publication of the international search report: 2 March 2000 (02.03.00)	
(72) Inventors; and (75) Inventors/Applicants (for US only): LUKACSOVICH, Tamas [HU/JP]; 2-30-13, Narusedai, Machida-shi, Tokyo 194-0043 (JP). ASZTALOS, Zoltan [HU/JP]; 3-16-21, Narusedai, Machida-shi, Tokyo 194-0043 (JP). YAMAMOTO, Daisuke [JP/JP]; 4-18-8, Narusedai, Machida-shi, Tokyo 194-0043 (JP). AWANO, Wakae [JP/JP]; Famiyu-102, 3-10-12, Minamidai, Sagami-hara-shi, Kanagawa 228-0814 (JP).			
(74) Agent: NISHIZAWA, Toshio; 6F, Mani-Building, 37-10, Udagawa-cho, Shibuya-ku, Tokyo 150-0042 (JP).			

(54) Title: A VECTOR FOR GENE TRAP, AND A METHOD FOR GENE TRAPPING BY USING THE VECTOR

## (57) Abstract

The present application provides a vector for trapping an unknown gene of *Drosophila melanogaster*, which is a recombinant plasmid comprising the following nucleotide sequences in this order: an artificial consensus splicing acceptor site; a synthetic "stop/start" sequence; a reporter gene; a drug resistance gene; a gene responsible for a detectable phenotype of the *Drosophila melanogaster*; and a synthetic splicing donor site. The present application also provides a method for trapping an unknown gene of *Drosophila melanogaster* by using the vector.



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Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/JP 99/02683

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/10 C12N15/62 C12N15/85 C12N15/90 C12Q1/68  
C07K14/435

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 20031 A (JARVIK JONATHAN W) 14 May 1998 (1998-05-14) the whole document ---	1-19
A	A.H. BRAND AND N. PERRIMON: "Targeted gene expression as a means of altering cell fates and generating dominant phenotypes" DEVELOPMENT, vol. 118, 1993, pages 401-415, XP000857179 THE COMPANY OF BIOLOGISTS, LIMITED, CAMBRIDGE, GREAT BRITAIN cited in the application the whole document --- -/--	1-19

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### Special categories of cited documents:

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- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

3 December 1999

Date of mailing of the international search report

22/12/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Hornig, H

# INTERNATIONAL SEARCH REPORT

Inte onal Application No  
PCT/JP 99/02683

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>W. WURST ET AL.: "A large-scale gene trap screen for insertional mutations in developmental mutations in developmentally regulated genes in mice"</p> <p>GENETICS, vol. 139, no. 2, February 1995 (1995-02), pages 889-899, XP000857167 GENETIC SOCIETY OF AMERICA, BALTIMORE, MD, US the whole document</p> <p style="text-align: center;">---</p>	1-19
A	<p>C. WILSON ET AL.: "P-element-mediated enhancer detection: an efficient method for isolating and characterizing developmentally regulated genes in Drosophila"</p> <p>GENES &amp; DEVELOPMENT, vol. 3, no. 9, September 1989 (1989-09), pages 1301-1313, XP000857178 CSH LABORATORY PRESS, NEW YORK, US cited in the application the whole document</p> <p style="text-align: center;">---</p>	1-19
A	<p>P. BARTHMAIER AND E. FYRBERG: "Monitoring development and pathology of Drosophila indirect flight muscles using green fluorescent protein"</p> <p>DEVELOPMENTAL BIOLOGY, vol. 169, no. 2, June 1995 (1995-06), pages 770-774, XP002124662 ACADEMIC PRESS, INC., US the whole document</p> <p style="text-align: center;">---</p>	1-19
A	<p>C.S. THUMMEL ET AL.: "Vectors for Drosophila P-element-mediated transformation and tissue culture transfection"</p> <p>GENE, vol. 74, 1988, pages 445-456, XP002124663 ELSEVIER SCIENCE PUBLISHERS, B.V., AMSTERDAM, NL; the whole document</p> <p style="text-align: center;">---</p>	
A	<p>V. PIRROTTA: "Vectors for P-mediated transformation in Drosophila"</p> <p>BIOTECHNOLOGY, VECTORS A SURVEY OF MOLECULAR CLONING VECTORS AND THEIR USES; R. L. RODRIGUEZ AND D.T. DENHARDT, vol. 1, 1988, pages 437-456, XP000857168 Butterworths, Boston, US cited in the application the whole document</p> <p style="text-align: center;">---</p>	
	-/--	

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/JP 99/02683

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>C.S. THUMMEL AND V. PIRROTTA: "New pCasPeR P element vectors" EMBL SEQUENCE DATABASE, 23 July 1996 (1996-07-23), XP002124664 Cambridge, UK Accession no. EMSYN.PEU59055; U59055; &amp; DROS. INFO. SERVICE, vol. 71, 1992, page 150 ---</p>	
A	<p>A. GOSSLER ET AL.: "Mouse embryonic stem cells and reporter constructs to detect developmentally regulated genes" SCIENCE, vol. 244, 1989, pages 463-465, XP002124665 AAAS, WASHINGTON, DC, US cited in the application the whole document -----</p>	

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/JP 99/02683

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9820031 A	14-05-1998	AU 5168598 A	29-05-1998



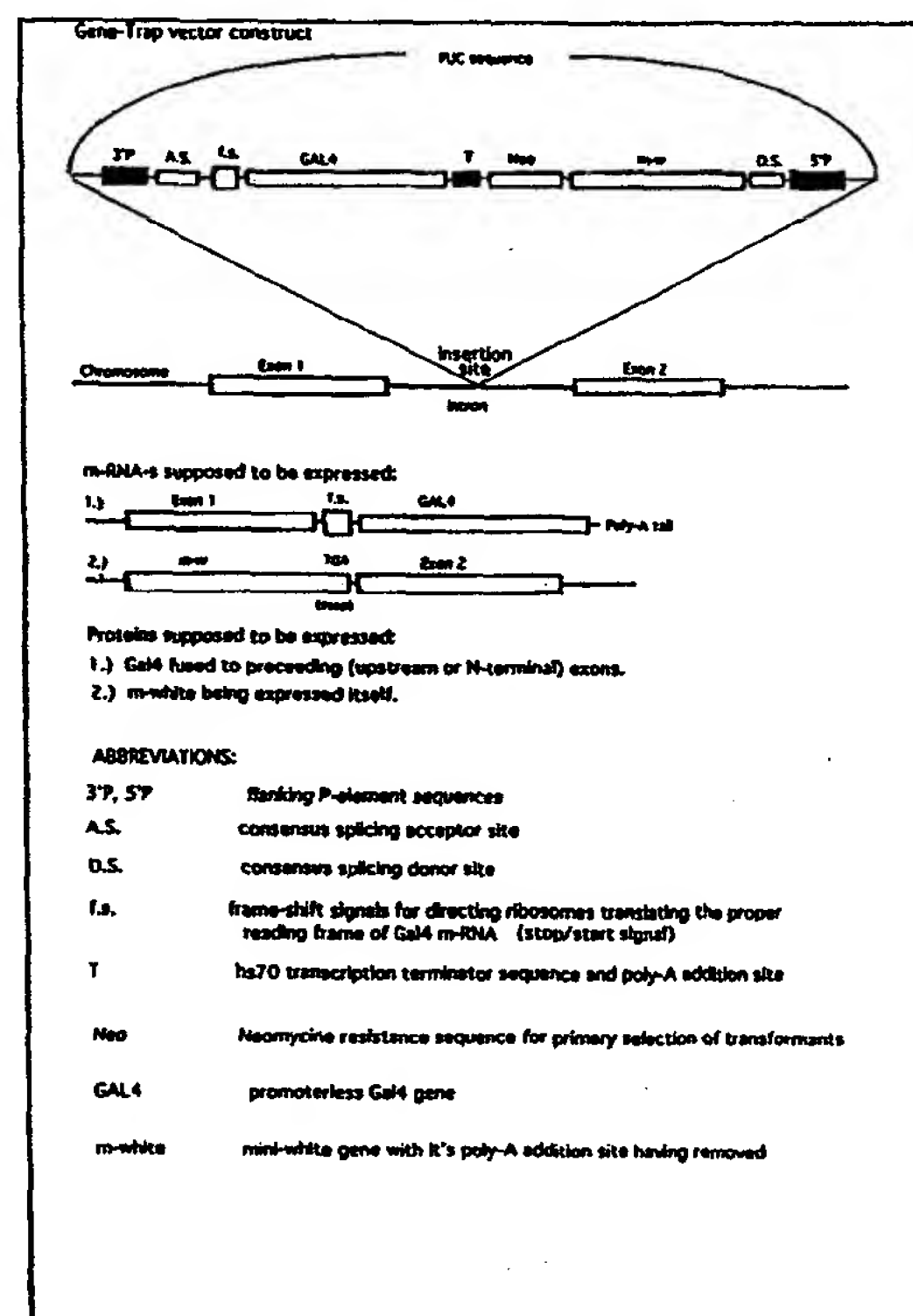
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: A VECTOR FOR GENE TRAP, AND A METHOD FOR GENE TRAPPING BY USING THE VECTOR

## (57) Abstract

The present application provides a vector for trapping an unknown gene of *Drosophila melanogaster*, which is a recombinant plasmid comprising the following nucleotide sequences in this order: an artificial consensus splicing acceptor site; a synthetic "stop/start" sequence; a reporter gene; a drug resistance gene; a gene responsible for a detectable phenotype of the *Drosophila melanogaster*; and a synthetic splicing donor site. The present application also provides a method for trapping an unknown gene of *Drosophila melanogaster* by using the vector.



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## Description

### A Vector for Gene Trap, and A Method for Gene Trapping by Using The Vector

5

## Technical Field

The present invention relates to a new vector system to facilitate the cloning and functional analysis of new genes of a fly, *Drosophila melanogaster*, and a method for gene trapping with the vector system.

## Background Art

There are numerous examples for application of gene trapping methods in wide range of living organisms including maize and mouse (Gossler et al., Science, 244:463-465, 1989).

With respect to tools for gene trapping, the application of different types of enhancer trap P-element vectors (Wilson et al., Genes & Development, 3:1301-1313, 1989) for cloning and analyzing trapped genes, as well their use for mosaic analysis with the help of the Gal4/UAS transcription activator system has proven fruitful. However, sometimes the expression pattern of the Gal4 or other reporter gene of the vector construct is affected by enhancers belonging to more than one gene. Similarly, in some cases it is difficult to determine whether the enhancer trap insertion effects the function of one or more of the neighboring genes.

These circumstances altogether with the fact that in some cases the mutant phenotype could be attributed to the

changed expression of a gene with its nearest exon located more than 30 kB apart from the insertion site, can lead in unfortunate cases to an ordeal when it's time to clone and analyze the affected gene.

5           One object of this application is to provide a vector system that includes specifically designed artificial regulatory sequences as well as selection methods for easy screening of positive recombinant lines. More especially, this application intends to provide a vector system of this  
10 invention offering much easier and faster cloning opportunities of the affected gene, compared to the widely used enhancer trap P-element vectors. Another object of this application is to provide easier detection method possibilities of the successful trapping events and much  
15 higher chance to get more characteristic ("functional") expression patterns of the reporter gene because in the contrary with much of the cases with enhancer trap lines, when using the vector system of this invention, the reporter gene expression is influenced only by a single endogenous  
20 transcription unit and effects only the expression of the very same gene.

### Disclosure of Invention

The first invention of this application is a vector for  
25 trapping an unknown gene of *Drosophila melanogaster*, which is a recombinant plasmid comprising the following nucleotide sequences in this order:

- an artificial consensus splicing acceptor site;
- a synthetic "stop/start" sequence;
- 30 a reporter gene;

a drug resistance gene;  
a gene responsible for a detectable phenotype of the *Drosophila melanogaster*; and  
a synthetic splicing donor site.

5 One embodiment of the first invention is that the recombinant plasmid is derived from pCasper3.

Other embodiments of the first invention are that the reporter gene is the Gal4 gene, Gal4 DNA binding domain-P53 fusion gene or the Gal4-firefly luciferase fusion gene.

10 Further embodiment of this first invention is that the gene responsible for a detectable phenotype of the *Drosophila melanogaster* is mini-white gene.

More further embodiment of the first invention is that the drug resistance gene is neomycin-phosphotransferase gene  
15 and its promoter is a heatshock promoter.

The second invention of this application is a method for trapping an unknown gene of *Drosophila melanogaster* by using a vector which is a recombinant plasmid comprising the  
20 following nucleotide sequences in this order:

an artificial consensus splicing acceptor site;  
a synthetic "stop/start" sequence;  
a reporter gene;  
a drug resistance gene;

25 a gene responsible for a detectable phenotype of the *Drosophila melanogaster*; and  
a synthetic splicing donor site,

which method comprises the steps of:

(a) introducing the vector into the genome of a white minus  
30 fly;

- (b) selecting primary transformants resistant to a drug;
- (c) crossing the primary transformants with a transposase source strain to force the vector to jump into other locations;
- 5 (d) selecting secondary transformants by picking up the flies having strong eye color,
- (e) crossing the secondary transformants with UAS (Upstream Activator Sequence)-luciferase harboring strain and measuring the reporter gene expression of the resultant flies; and
- 10 (f) identifying the trapped gene by cloning and sequencing the cDNAs fused to the reporter gene and the gene responsible for a detectable phenotype of the fly.

The third invention of this application is a method for  
15 trapping an unknown gene of *Drosophila melanogaster* by using a vector A which is a recombinant plasmid comprising the following nucleotide sequences in this order:

- an artificial consensus splicing acceptor site;
  - a synthetic "stop/start" sequence;
  - 20 Gal4 DNA binding domain-P53 fusion gene as a reporter gene;
  - a drug resistance gene;
  - a gene responsible for a detectable phenotype of the *Drosophila melanogaster*; and
  - a synthetic splicing donor site,
  - 25 and a vector B derived from pCasperhs, which has the heatshock promoter directed Gal4 activator domain-large T antigen fusion gene within polycloning site of the pCasperhs,
- which method comprises the steps of:
- (a) introducing each of the vectors A and B into the  
30 genomes of separate white minus flies;

(b) selecting primary transformants for the vector A which are resistant to the drug, and selecting primary transformants for the vector B which have an eye color;

(c) crossing the primary transformants for the vector A  
5 with a transposase source strain to force the vector to jump into other locations;

(d) selecting secondary transformants for the vector A by picking up the flies having strong eye color;

(e) crossing the secondary transformants with the primary  
10 transformants for the vector B to obtain flies harboring both the vectors A and B;

(f) crossing the flies obtained in the step (e) with an UAS-luciferase harboring fly strain and measuring the reporter gene expression of the resultant flies after a  
15 heatshock treatment; and

(g) identifying the trapped gene by cloning and sequencing the cDNAs fused to the reporter gene and the gene responsible for a detectable phenotype of the fly.

20       Embodiments of the second and third inventions are corresponded to the embodiments of the first invention, and they will be more precisely described in the following description.

## 25                   Brief Description of Drawings

Figure 1 shows the schematic map of the vector of this invention, pTrap-hsneo.

Figure 2 shows the schematic map of the vector of this invention, pTrap-G4-p53.

30       Figure 3 shows the schematic map of the vector of this

invention, pCasperhs-G4-LT.

Figure 4 shows the schematic map of the vector of this invention, pTrap-G4-luc.

Figure 5 shows the schematic drawing of a fly genome to which the vector of this invention is inserted for cloning.

Figure 6 shows the results of sequencing RT-PCR products of aop-Gal4 and m-white-aop fusion mRNAs.

Figure 7 presents pictures of characteristic beta-galactosidase staining patterns in different parts of the fly brain resulted from crossing positive gene trap lines with flies harboring a UAS-lacZ construct.

#### Best Mode for Carrying Out the Invention

A vector construct of the first invention, for example, can be based on the commonly used, P-element transformation vector, pCasper3 (Pirotta, Vectors: A survey of molecular cloning vectors and their uses, eds. Rodriguez, R.L. & Denhardt, D.T., Butterworths, Boston. 437-456, 1998) and the convenient Gal4-UAS expression system (Brand and Perrimon, Development, 118:401-415, 1993).

A promoterless Gal4 gene preceded by an artificial consensus splicing acceptor site and a synthetic "stop/start" sequence to govern the read through translation coming from upstream exon(s) of the trapped gene into the proper reading frame of Gal4 was inserted into the polycloning site of pCasper3.

The removal of the whole 3' UTR (untranslated region) sequence of the mini-white gene and replacement by an artificial splicing donor site resulted in a truncated gene without its own poly-adenylation site.

Without a successful gene trapping event this truncated mini-white gene was not expected to confer any eye color, therefore in this invention a heatshock promoter directed neomycin-phosphotransferase (hs-neo) gene for helping  
5 selection of primary transformants by antibiotic feeding has been inserted.

Figure 1 shows the schematic map of the gene trap construct (pTrap-hsneo), and SEQ ID No.1 is the complete nucleotide sequence of the vector pTrap-hsneo.

10 Another gene trap construct, pTrap-G4-p53 (Figure 2) is created by replacing the Gal4 coding sequence of plasmid pTrap-hsneo with a Gal4 DNA binding domain-P53 fusion gene (Clontech, Matchmaker Two Hybrid System, #K1605-1). When this construct coexists in the genome of the same fly with another  
15 vector, pCasperhs-G4-LT (Figure 3) containing a heatshock promoter directed Gal4 activator domain-large T antigen (Clontech, Matchmaker Two Hybrid System, #K1605-1) fusion gene, the assembly of a functional Gal4 molecule, through p53-large T antigen interaction, can be regulated by external  
20 heatshock.

On this way, the possibility of an intentional temporary control of Gal4 activity became available. In other words the Gal4 expression in a pattern as already determined spatially by the promoter of the trapped gene now can be  
25 induced at any desired stage of development by external heatshock.

In order to make the detection of Gal4 expression easier, the Gal4 gene in another construct is replaced with a Gal4-firefly luciferase fusion gene to get pTrap-G4-luc  
30 (Figure 4). This artificial gene is coding for a fusion

polypeptide which has preserved both enzymatic activities.

The easy measuring of luciferase activity by luminoassay (Brandes et al., Neuron, 16:687-694, 1996) makes the detection of Gal4 activity comfortable in every single  
5 living fly.

Then, one of the best mode of the second or third invention, a method for gene trapping using the vector system, is described in detail.

10 (1) Screening:

The gene trap vector constructs can be introduced into the genome of a white minus fly by microinjection. The selection of primary transformants is possible by using G418, an analog of neomycin, resistance conferred by hs-neo gene.  
15 (When performing transformation experiments with these constructs it's turned out that the truncated mini-white gene generally provides a very slight yellow eye color which could be distinguished from w-minus phenotype in most of the cases, therefore G418 selection apparently is not necessary.)

20 After a line with the gene trap construct is being established, the secondary transformants can be generated on the usual way by crossing the original line with a so-called jumpstarter containing the transposase expressing delta 2-3 genetic element.

25 Usually a certain percentage, between 4 and 8, of the secondary transformants have much stronger eye color (deep orange or reddish) than the ancestor fly indicating that the construct was being inserted downstream of a promoter and now the mini-white gene is using the transcriptional "facilities"  
30 of that gene (e.g.: poly-adenylation site and transcriptional



terminator) instead of its removed ones. They are the most likely candidates for successful gene trap events. In case of these lines the vector probably has been inserted either into an intron of a gene or upstream from the first intron into the 5' UTR in proper orientation (that is the direction of transcription is same for the "trapped gene" and the mini-white (and Gal4) genes as well). The mini-white gene has its own promoter therefore its expression pattern is supposed to be largely independent from that of the trapped gene.

10           These positive lines are to be checked in the next step for Gal4 expression by crossing them with a "marker" line harboring a UAS-luciferase reporter gene construct. (When using pTrap-G4-luc vector, this step is obviously not necessary.) Usually very strong correlation was found between  
15 eye color and Gal4 expression: more than 90% of the lines having strong eye color proved to be expressing Gal4 by means of luciferase assay using luminometer (Brandes et al., Neuron, 16:687-692, 1996).

20   (2) Cloning:

          When the gene trap construct is being inserted into an intron of an endogenous gene, the marker genes of the construct are supposed to be spliced on mRNA level to the exons of the trapped gene by using the artificial splicing  
25 acceptor and donor sites. More exactly while the Gal4 mRNA should be joint to the exon(s) located upstream of the insertion site, at the same time the mini-white mRNA is fused to the following exon(s) accomplishing the dual tagging of the trapped gene (Figure 5).

30           This feature can be used for quickly and easily

identifying the trapped gene by means of 3' and 5' RACE  
(Rapid Amplification of cDNA Ends) experiments. Even cloning  
and sequencing only a part of the caught mRNA still provides  
reasonable chance to find homologous mRNAs in the BDGP  
5 (Berkeley Drosophila Genome Project) EST (Expressed Sequence  
Tag) library.

With these approaches, the identification of an already  
cloned gene can take less than a week compared to the usually  
more than one year period in average when analyzing a mutant  
10 created by some enhancer trap construct.

It's well-known from the literature and the present  
inventors also have experienced that P-element vectors tend  
to integrate into or near the 5' UTR of active genes. (The  
present inventors found that in these cases if the insertion  
15 occurred upstream from the first intron, and therefore the  
artificial splicing acceptor site could not be utilized, the  
Gal4 gene was expressed by read-through transcription from  
the nearby promoter.)

The advantage of this tendency can be taken by cloning  
20 and sequencing the flanking genomic sequences of the  
insertion site by inverse or vectorette PCR or by plasmid  
rescue using suitable restriction digestion to recover the  
neomycin resistance gene of the construct. Then again the  
BDGP library can be searched to find any significant matching.

25

### (3) Rescue:

The only reliable way to confirm that any observed  
mutant phenotype is really the consequence of the P-element  
insertion is to rescue that particular phenotype. Expectedly  
30 the phenotype (some alteration from wild type fly) is caused

by changed expression of gene(s) disturbed by insertion of the P-element. The rescue can be made by expressing the cDNA of the suspected gene most preferable with identical spatial and temporary pattern than that of the gene itself.

5           As it was expected, the vector constructs of the first invention usually cause strong phenotypes. It's not surprising at all because the trapped genes are supposed to be split into two parts on mRNA level resulting in null mutants in majority of the cases. Accordingly mutants  
10 obtained by this method frequently show homozygous lethality or sterility. Hypomorphic mutants can be obtained by forcing imprecise excision of the gene trap P-element construct.

          As mentioned above, the Gal4 expression is obliged to reflect precisely to that of the trapped gene simply because  
15 the Gal4 gene has no its own promoter and they share a common, fused mRNA.

          This identical expression provides unique opportunity to rescue the mutant phenotype by crossing this fly with another one harboring the UAS directed, cloned cDNA of the  
20 trapped gene.

          On this way either the original, homozygous null mutant gene trap fly or any transheterozygous derivative of that with some hypomorphic allele over the null mutant allele can be rescued.

25

(4) Determination of spatial and developmental expression pattern of the trapped gene:

          Histochemical determination of the spatially and temporarily controlled expression of any trapped gene is also  
30 easy following introduction of a UAS-lacZ construct into the

genome of the same fly and performing either X-gal or antibody staining for beta-galactosidase.

(5) Mosaic analysis:

Possession of a large collection of fly lines with  
5 different, characteristic and, in the case of the pTrap-G4-  
p53/pCasperhs-G4-TL vector system, inducible Gal4 expression  
pattern makes feasible carrying out mosaic analysis of  
virtually any gene of interest by directing the expression of  
their UAS-constructs on a mutant background with different  
10 Gal4 expression patterns.

This approach can answer the question of where and when  
that particular gene is required to be expressed to rescue  
the mutant phenotype.

Similarly, any gene can be expressed in different  
15 ectopic patterns to generate new dominant mutant phenotypes.  
This approach might help to conclude the role of that  
particular gene and to identify the pathway, in which it's  
involved.

20

Example

The following example illustrates a specific embodiment  
of the various aspects of the invention. This example is not  
intended to limit the invention in any manner.

Figure 6 shows the results of sequencing RT-PCR  
25 products of aop-Gal4 and m-white-aop fusion mRNAs.

The template was total RNA prepared from a positive  
gene trap line which has the vector pTrap-hsneo being  
integrated into the first intron of the well-known aop  
(anterior open/pokkuri/yan) developmental gene. The sequences  
30 confirm that both splicing occurred precisely at that

particular nucleotides of the artificial regulatory sequences where it was expected.

On Figure 7, there are pictures of characteristic beta-galactosidase staining patterns in different parts of the fly  
5 brain resulted from crossing positive gene trap lines with flies harboring a UAS-lacZ construct.

### Industrial Applicability

The vector system of this invention offers an  
10 exceptional opportunity for easy and fast cloning of the gene responsible for the observed phenotype. Furthermore, by using the UAS-driven coding sequence of any gene of interest, that particular gene can be expressed in identical patterns than those of the trapped genes and these expressions can be  
15 regulated temporarily at any desired developmental stage.

### Sequence Listing

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20 by Using The Vector  
<150> Japan, Application No. 10-141952  
<151> 22 May 1998  
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- 20 <221> mini-white gene  
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## CLAIMS

1. A vector for trapping an unknown gene of *Drosophila melanogaster*, which is a recombinant plasmid comprising the  
5 following nucleotide sequences in this order:  
an artificial consensus splicing acceptor site;  
a synthetic "stop/start" sequence;  
a reporter gene;  
a drug resistance gene;  
10 a gene responsible for a detectable phenotype of the *Drosophila melanogaster*; and  
a synthetic splicing donor site.
2. The vector of claim 1, wherein the recombinant plasmid  
15 is derived from pCasper3.
3. The vector of claim 1 or 2, wherein the reporter gene is the Gal4 gene.
- 20 4. The vector of claim 3, which has the nucleotide sequence of SEQ ID No. 1.
5. The vector of claim 1 or 2, wherein the reporter gene is Gal4 DNA binding domain-P53 fusion gene.
- 25 6. The vector of claim 1 or 2, wherein the reporter gene is the Gal4-firefly luciferase fusion gene.
7. The vector of any one of claims 1-6, wherein the gene responsible for a detectable phenotype of the *Drosophila*  
30 *melanogaster* is mini-white gene.

8. The vector of any one of claims 1-7, wherein the drug resistance gene is neomycin-phosphotransferase gene and its promoter is a heatshock promoter.

5

9 A vector derived from pCasperhs, which has the heatshock promoter directed Gal4 activator domain-large T antigen fusion gene within polycloning site of the pCasperhs.

10 10. A method for trapping an unknown gene of *Drosophila melanogaster* by using a vector which is a recombinant plasmid comprising the following nucleotide sequences in this order:

an artificial consensus splicing acceptor site;

a synthetic "stop/start" sequence;

15 a reporter gene;

a drug resistance gene;

a gene responsible for a detectable phenotype of the *Drosophila melanogaster*; and

a synthetic splicing donor site,

20 which method comprises the steps of:

(a) introducing the vector into the genome of a white minus fly;

(b) selecting primary transformants resistant to a drug;

(c) crossing the primary transformants with a transposase  
25 source strain to force the vector to jump into other locations;

(d) selecting secondary transformants by picking up the flies having strong eye color,

(e) crossing the secondary transformants with UAS (Upstream  
30 Activator Sequence)-luciferase harboring strain and measuring



the reporter gene expression of the resultant flies; and

(f) identifying the trapped gene by cloning and sequencing the cDNAs fused to the reporter gene and the gene responsible for a detectable phenotype of the fly.

5

11. The method according to claim 10, wherein the recombinant plasmid is derived from pCasper3.

12. The method according to claim 10 or 11, wherein the  
10 reporter gene in the vector is the Gal4 gene, and in the step (e) the Gal4 expression is measured.

13. The method according to claim 10 or 11, wherein the  
15 reporter gene of the vector is the Gal4-firefly luciferase fusion gene, and in the step (e) expression of said fusion gene is measured without crossing the secondary transformants with UAS-luciferase harboring strain.

14. The method according to any one of claims 10 to 14,  
20 wherein the gene responsible for a detectable phenotype of the *Drosophila melanogaster* is mini-white gene, and in the step (f) the cDNAs fused to the reporter gene and the mini-white gene are cloned and sequenced.

15. The method according to any one of claims 10 to 15,  
25 wherein the drug resistance gene is neomycin-phosphotransferase gene and its promoter is a heatshock promoter, and in the step (b) the transformants resistant to G418 is selected.

30 16. A method for trapping an unknown gene of *Drosophila*



*melanogaster* by using a vector A which is a recombinant plasmid comprising the following nucleotide sequences in this order:

- an artificial consensus splicing acceptor site;
  - 5 a synthetic "stop/start" sequence;
  - Gal4 DNA binding domain-P53 fusion gene as a reporter gene;
  - a drug resistance gene;
  - a gene responsible for a detectable phenotype of the *Drosophila melanogaster*; and
  - 10 a synthetic splicing donor site,
- and a vector B derived from pCasperhs, which has the heatshock promoter directed Gal4 activator domain-large T antigen fusion gene within polycloning site of the pCasperhs, which method comprises the steps of:
- 15 (a) introducing each of the vectors A and B into the genomes of separate white minus flies;
  - (b) selecting primary transformants for the vector A which are resistant to a drug, and selecting primary transformants for the vector B which have an eye color;
  - 20 (c) crossing the primary transformants for the vector A with a transposase source strain to force the vector to jump into other locations;
  - (d) selecting secondary transformants for the vector A by picking up the flies having strong eye color;
  - 25 (e) crossing the secondary transformants with the primary transformants for the vector B to obtain flies harboring both the vectors A and B;
  - (f) crossing the flies obtained in the step (e) with an UAS-luciferase harboring fly strain and measuring the
  - 30 reporter gene expression of the resultant flies after a

heatshock treatment; and

(g) identifying the trapped gene by cloning and sequencing the cDNAs fused to the reporter gene and the gene responsible for a detectable phenotype of the fly.

5

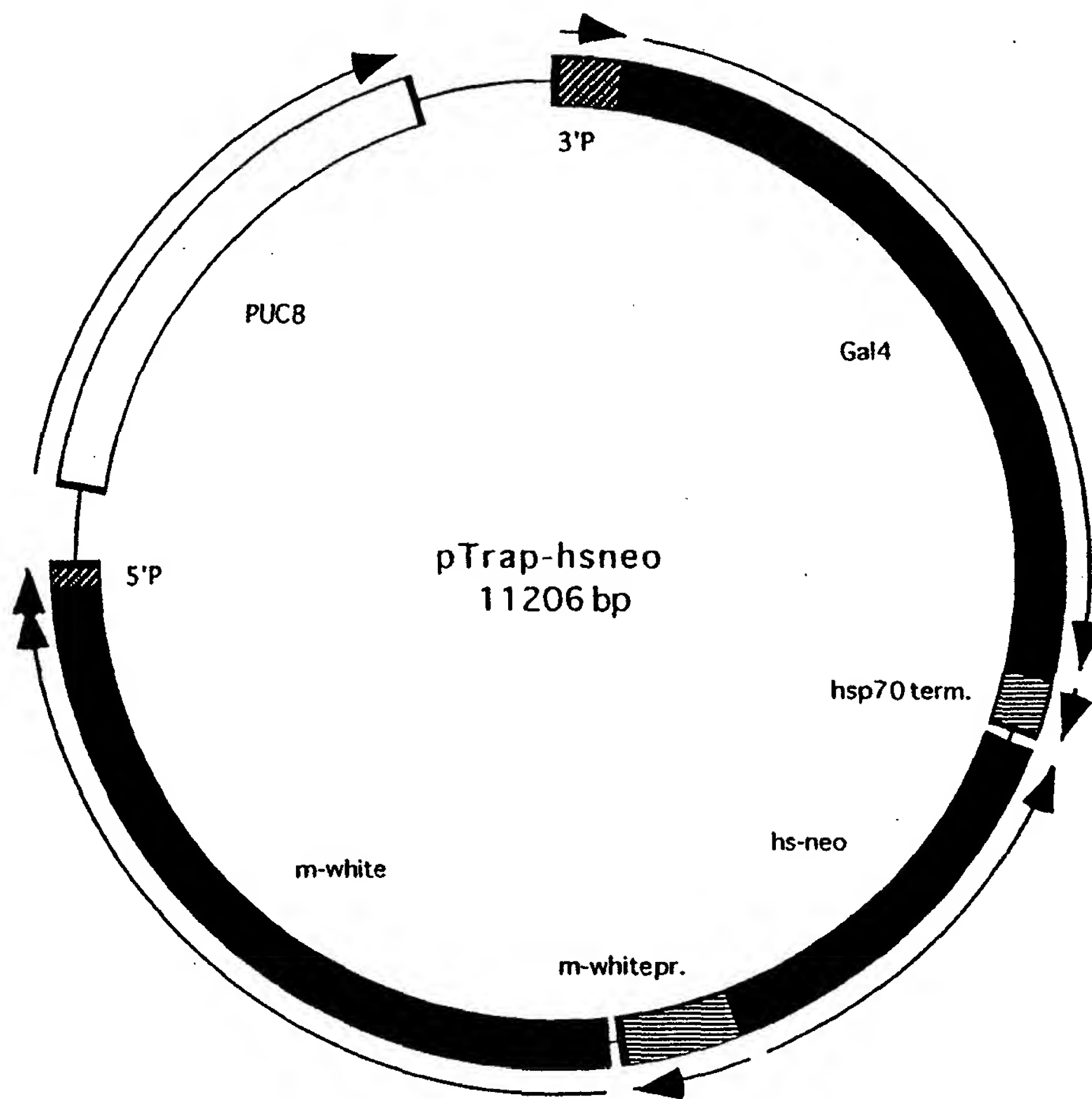
17. The method according to claim 16, wherein the vector A is derived from pCasper3.

18. The method according to claim 16 or 17, wherein the  
10 gene responsible for a detectable phenotype of the *Drosophila melanogaster* is mini-white gene, and in the step (g) the cDNAs fused to the reporter gene and the mini-white gene are cloned and sequenced.

15 19. The method according to any one of claims 16 to 18, wherein the drug resistance gene is neomycin-phosphotransferase gene and its promoter is a heatshock promoter, and in the step (b) the transformant resistant to G418 is selected.

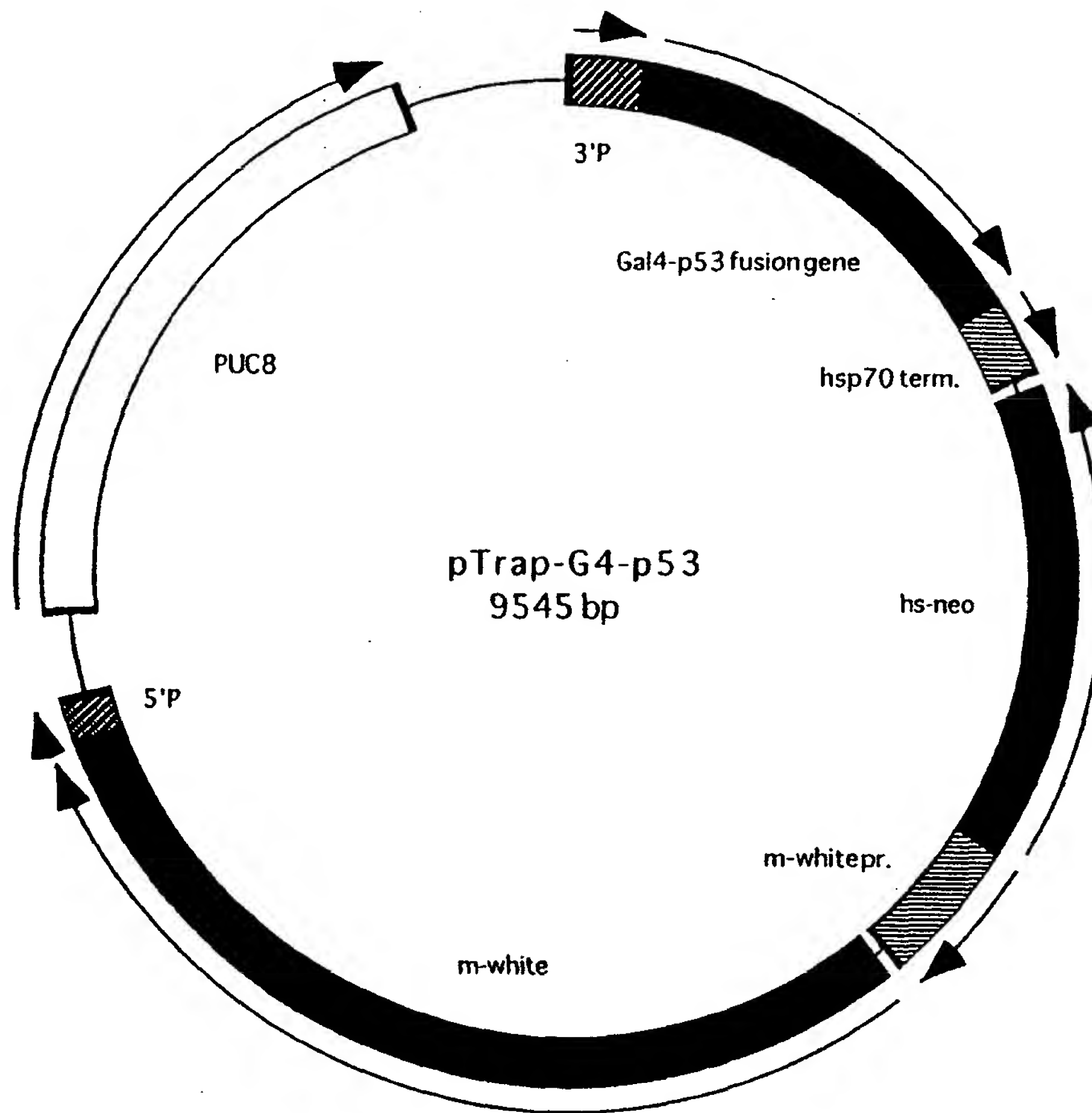
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Fig. 1



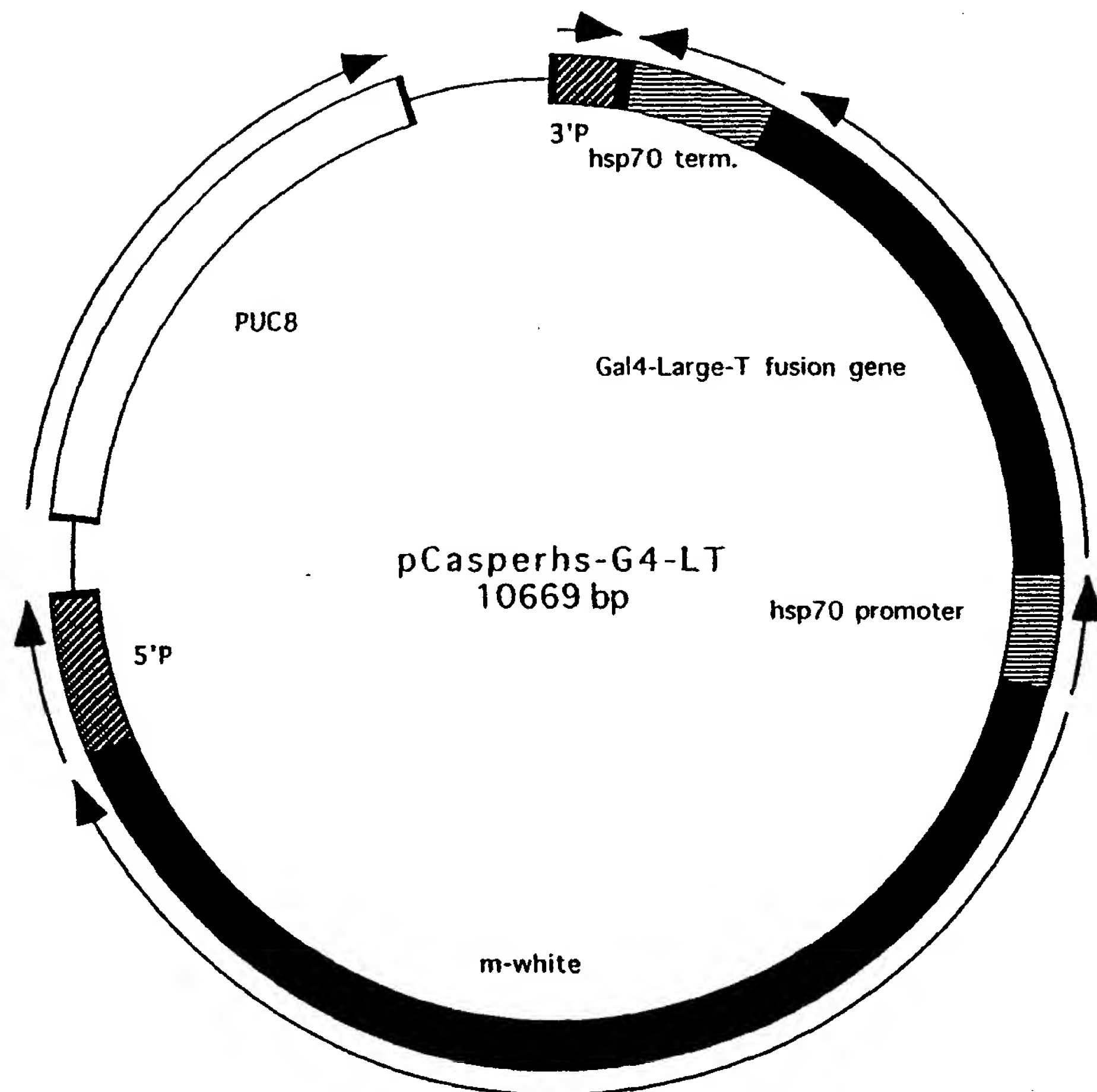
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Fig. 2



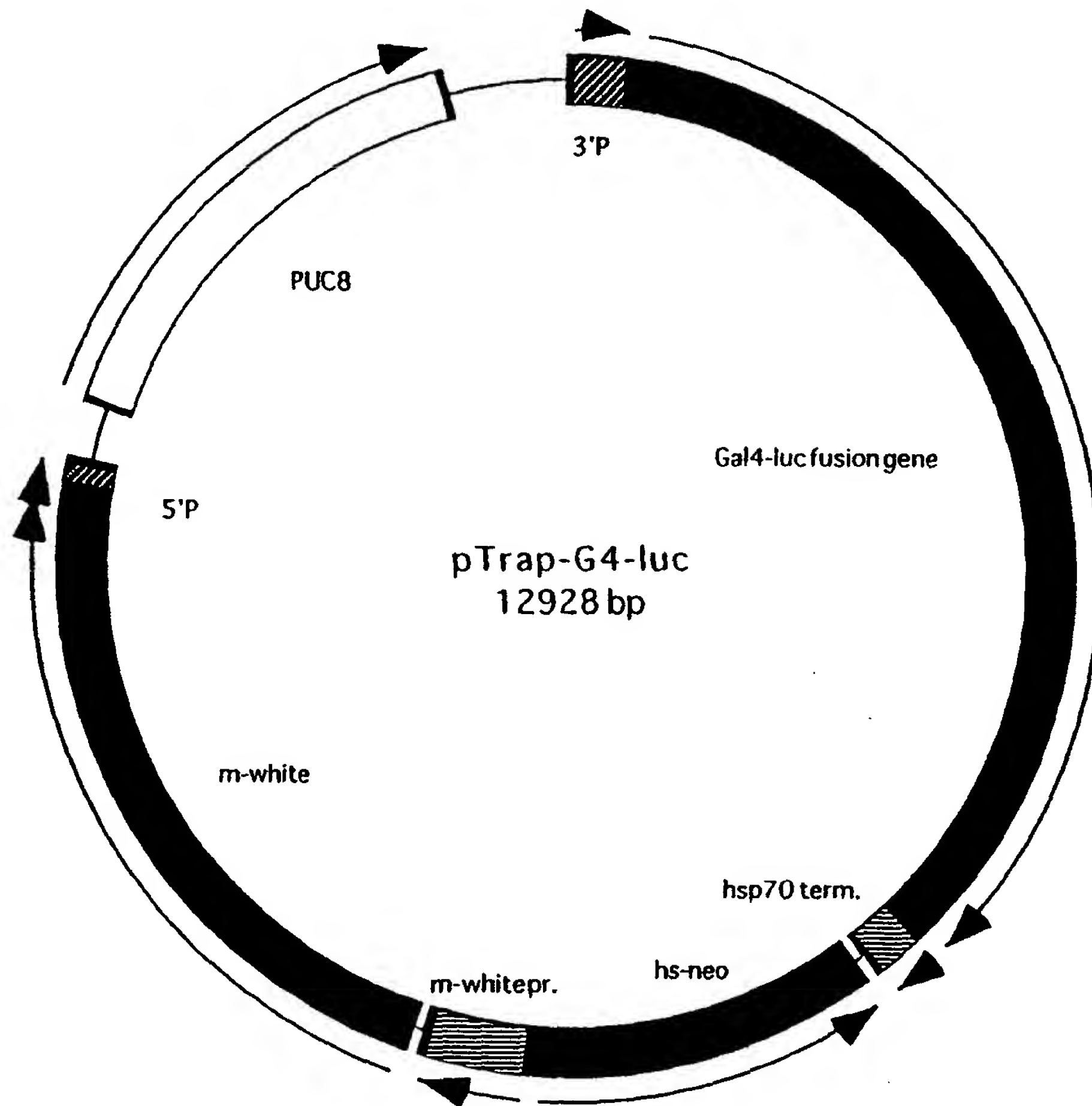
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Fig. 3



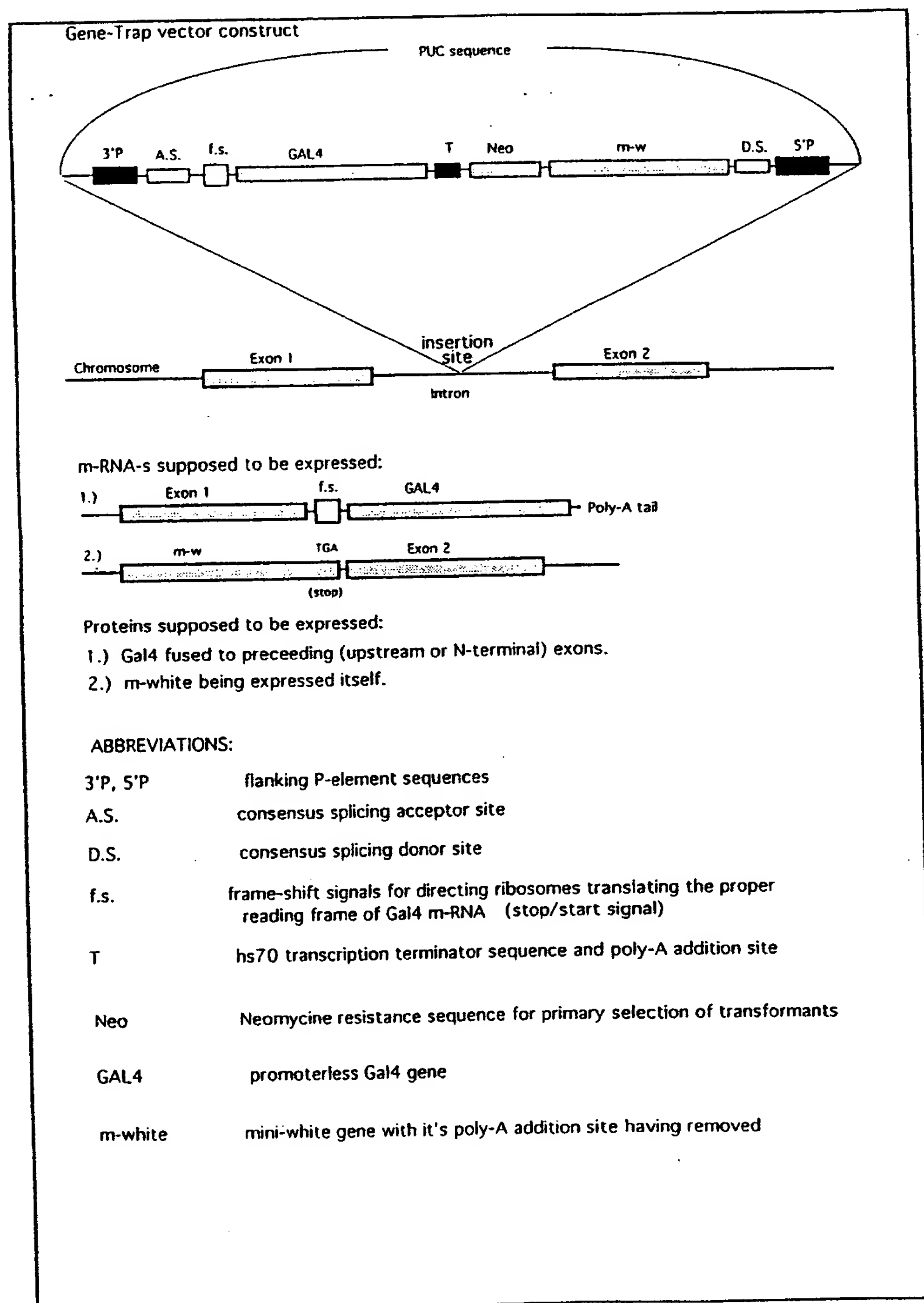
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Fig. 4



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Fig. 5



6/7

*Fig. 6*

Precise splicing of Gal4 and mini-white genes from Gene Trap vector  
into anterior open gene

5'P end of vector; splice acceptor site/stop-start seq./Gal4 gene.....mini-white gene/splice donor site; 3'P end of GT vector  
.....<http://cagtagtaagaaagtctttcttctaacg/gtcgaattgtatgtatgatcatgaatgcot.....agaacttggtcccgacagaggtagtagag/gtaagtagccggccgacctaaaggttatatg>

anterior open circuit exon 1 exon 2  
cagtagctaacacacagtgcacagtggaacaacag/atatacacggttaaaagcaattccagtggttccac

**anterior open exon 1 - Gal4 fusion cDNA**

```
cagtacgtaaacacagtcacagtgagcgaacag/stoyattgttgttggatccaatggagct
```

mini-white - anterior open exon 2 fusion cDNA  
 agactctggggccgacgacgagtagag/ataacacgcgcttaaaagcaattccagtggtctccac



Gal4 expression patterns revealed by UAS-lacZ reporter construct.

Fig. 7

